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cosmetic and quasi-drug research group

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Prohibited substances

# Analytical Methods of Cosmetic Ingredients in Cosmetic Products

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MINISTRY OF FOOD AND DRUG SAFETY
National Institute
of Food and Drug Safety Evalution

Pharmaceutical and Medical Device Research Department
Cosmetics Research Division

This document aims to provide the cosmetic industry with information regarding the analysis of cosmetic ingredients present in cosmetic products. The analytical methods in this document were validated by research funded by the Ministry of Food and Drug Safety (MFDS) of the Republic of Korea. Before adopting the analytical methods according to the types of cosmetic, it is recommended to validate the methods with suitable procedures. If the analytical methods are modified, the modified methods should be evaluated in advance with appropriate validation procedures.

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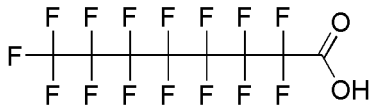
# 1 Per- and polyfluorinated substances(PFAS)

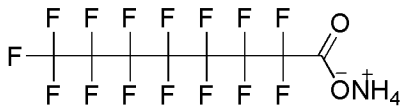
## Chemical information

### Perfluorooctanoic acid(PFOA)



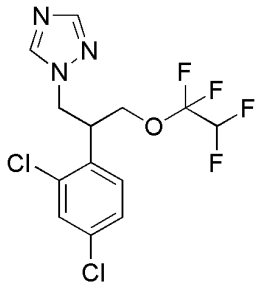
- Molecular formula: $C_8HF_{15}O_2$
- Molecular weight: 414.07
- CAS number: 335-67-1

### Ammonium pentadecafluorooctanoate Pentadecafluorooctanoic acid ammonium salt



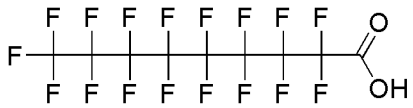
- Molecular formula: $C_8H_4F_{15}NO_2$
- Molecular weight: 431.10
- CAS number: 3825-26-1

## Tetraconazole



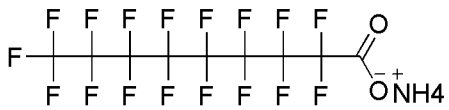
- Molecular formula: $C_{13}H_{11}Cl_2F_4N_3O$
- Molecular weight: 372.15
- CAS number: 112281-77-3

## Perfluorononanoic acid(PFNA)



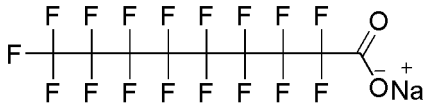
- Molecular formula: $C_9HF_{17}O_2$
- Molecular weight: 464.08
- CAS number: 375-95-1

## Ammonium perfluorononanoate



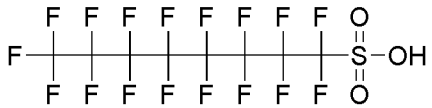
- Molecular formula: $C_9H_4F_{17}NO_2$
- Molecular weight: 481.11
- CAS number: 4149-60-4

## Sodium heptadecafluorononanoate



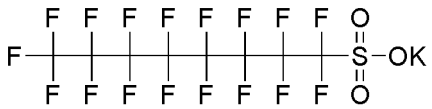
- Molecular formula: $C_9F_{17}NaO_2$
- Molecular weight: 486.06
- CAS number: 21049-39-8

## Perfluorooctanesulfonic acid(PFOS)



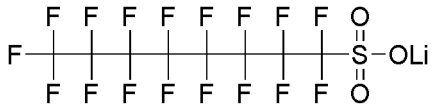
- Molecular formula: $C_8HF_{17}O_3S$
- Molecular weight: 500.13
- CAS number: 1763-23-1

## Potassium perfluorooctanesulfonate



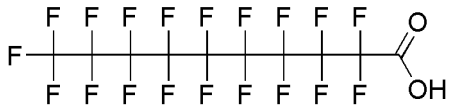
- Molecular formula: $C_8F_{17}KO_3S$
- Molecular weight: 538.22
- CAS number: 2795-39-3

## Lithium perfluorooctanesulfonate



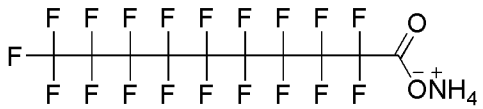
- Molecular formula: $C_8F_{17}LiO_3S$
- Molecular weight: 506.06
- CAS number: 29457-72-5

## Nonadecafluorodecanoic acid



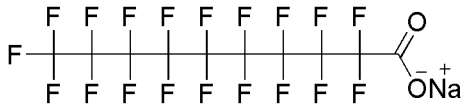
- Molecular formula: $C_{10}HF_{19}O_2$
- Molecular weight: 514.08
- CAS number: 335-76-2

## Ammonium nonadecafluorodecanoate



- Molecular formula: $C_{10}H_4F_{19}NO_2$
- Molecular weight: 531.11
- CAS number: 3108-42-7

## Sodium nonadecafluorodecanoate



- Molecular formula: $C_{10}F_{19}NaO_2$
- Molecular weight: 536.07
- CAS number: 3830-45-3

## Assay

- General Precautions

  Due to the potential impact of laboratory glassware on the analysis results of per- and polyfluoroalkyl substances(PFAS), use containers made of appropriate materials, such as polypropylene.

- Procedure

  Preparation of sample solution - Powders

  In a 10-mL volumetric flask, weigh 1.0 g of the sample, and make it up to 5 mL using methanol. Sonicate the mixture to disperse the sample evenly, make it up to 10 mL using the methanol, and centrifuge this solution. Transfer 5 mL of supernatant, add 1 mL of the internal standard solution, and make it up to 10 mL using methanol. Pass the resulting solution through a suitable filter of 0.2-μm pore size(excluding potentially contaminated filters such as PTFE* membrane), and use this solution as the sample solution.

  * PTFE : Poly tetra fluoro ethylene

### Preparation of sample solution – Creams

In a 10-mL volumetric flask, weigh 1.0 g of the sample, and make it up to 5 mL using methanol. Sonicate the mixture to disperse the sample evenly, make it up to 10 mL using the methanol, and centrifuge this solution. Transfer 5 mL of supernatant and make it up to 10 mL using methanol. Pass the resulting solution through a suitable filter of 0.2-μm pore size(excluding potentially contaminated filters such as PTFE* membrane). Precondition a Prime HLB cartridge(6 cc, 500 mg) or equivalent cartridge with 3 mL of methanol, followed by 3 mL of water, discarding both washings. Load 1 mL of the filtrated solution onto the cartridge, apply suction until the solution reaches 1-2 mm above the upper part of the cartridge's stationary phase, and elute with 4 mL of 5% methanol, followed by 4 mL of methanol. Evaporate the collected solution in water bath of below 40℃ under nitrogen gas. Dissolve the residue with 0.1 mL of the internal standard stock solution and 0.9 mL of methanol and use this solution as the sample solution.

* PTFE : Poly tetra fluoro ethylene

### Preparation of standard solution

In a 20-mL volumetric flask, weigh 20 mg of the per- and poly-fluorinated substnaces, make it up to 20 mL using methanol, and use this solution as the standard stock solution(1,000 μg/mL). Dilute appropriate volume of each standard stock solution with methanol to make the mixed standard stock solution(1 μg/mL). In a 6 different 10-mL volumetric flasks, transfer 0.05, 0.1, 0.2, 0.25, 0.5, and 1 mL of the mixed stadard stock solution, respectively, add 1 mL of the internal standard solution to each flask, make it up to 10 mL using methanol, and use this solution as the standard solution.

Analyze the prepared standard solutions and sample solution as directed under liquid chromatography triple quadrupole-mass spectrometry. The sample amount or dilution factor may be adjusted within the range of the calibration curve, if necessary.

*Internal standard solution: Dilute each internal stadard with methanol to make the internal standard stock solution(10 ng/mL).

※ Internal standard: Perfluoro-n-[1,2,3,4-13C4]octanoic acid,
Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid
Sodium perfluoro-1-[1,2,3,4-13C4]octanesulfonate
Perfluoro-n-[1,2-13C2]dodecanoic acid

- **Chromatographic system**

**Mode:** Liquid chromatography-triple quadrupole mass spectrometry

**Column:** ACQUITY UPLC BEH C18 Column (2.1 x 100 mm, 1.7 μm) or equivalent column

**Column temperature:** 35℃

**Isolator Column:** Atlantis Premier BEH C18 AX Column(2.1 x 50 mm, 5.0 μm) or equivalent column

**Mobile phase:** (A) 2 mM Ammonium acetate in water
(B) 2 mM Ammonium acetate in methanol

| Time | A (%) | B (%) |
|---|---|---|
| 0 | 95 | 5 |
| 1 | 75 | 25 |
| 6 | 50 | 50 |
| 13 | 15 | 85 |
| 14 | 5 | 95 |
| 17 | 5 | 95 |
| 20 | 95 | 5 |

**Flow rate:** 0.3 mL/min

**Injection volume:** 2 μL

**Mutiple reaction monitoring(MRM):**

| Name | Ion Mode | Exact mass | Precursor ion | Cone Voltage | Product ions | Collision Energy |
|---|---|---|---|---|---|---|
| Perfluorooctanoic acid and its salts(ammonium salt) | − | 413.97 | 412.9 | 13 | 368.7 | 10 |
| | | | | | 168.8 | 18 |
| Tetraconazole | + | 371.02 | 371.7 | 30 | 158.7 | 32 |
| | | | | | 70.1 | 22 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Perfluorononanoic acid and its salts(ammonium, sodium salt) | − | 463.97 | 462.8 | 13 | 418.7 | 10 |
| | | | | | 218.8 | 17 |
| Perfluorooctanesulfonic acid and its salts(potassium, lithium salt) | − | 499.94 | 498.7 | 48 | 80.0 | 46 |
| | | | | | 98.9 | 40 |
| Nonadecafluorodecanoic acid and its salts(ammonium, sodium salt) | − | 513.97 | 512.8 | 13 | 468.7 | 12 |
| | | | | | 268.7 | 18 |
| Perfluoro−n−[1,2,3,4−13C4]octanoic acid(Perfluorooctanoic acid internal standard) | − | 417.99 | 416.8 | 13 | 371.9 | 10 |
| Perfluoro−n−[1,2,3,4,5−13C5]nonanoic acid(Perfluorononanoic acid internal standard) | − | 468.99 | 467.8 | 13 | 422.8 | 10 |
| Sodium perfluoro−1−[1,2,3,4−13C4]octane sulfonate(Perfluorooctanesulfonic acid internal standard) | − | 525.93 | 502.8 | 10 | 79.9 | 42 |
| Perfluoro−n−[1,2−13C2]dodecanoic acid(Nonadecafluorodecanoic acid internal standard) | − | 515.97 | 514.8 | 10 | 469.9 | 12 |

* The conditions for multiple reaction monitoring(MRM) can be altered by variations in instrument status and solvent conditions.

Calculate the percentage amount of each ingredients using the following expression:

$$\text{Amount (\%) of each ingredients} = C \times \frac{V}{S \times 10000} \times D$$

C : Concentration of each ingredients calculated from calibration curve (ng/mL)

V : Final volume of the sample solution (mL)

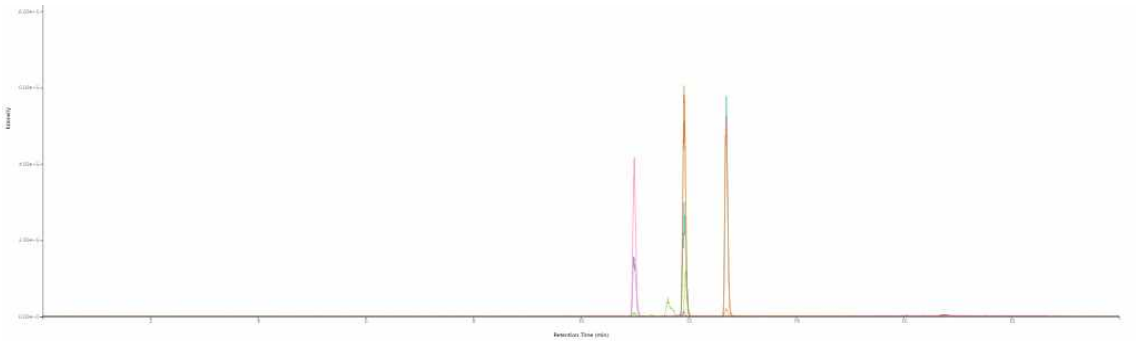
S : Amount of sample (g)

10000 : Conversion factor

D : Dilution factor

# Chromatogram & Spectrum

- Total Ion Chromatogram



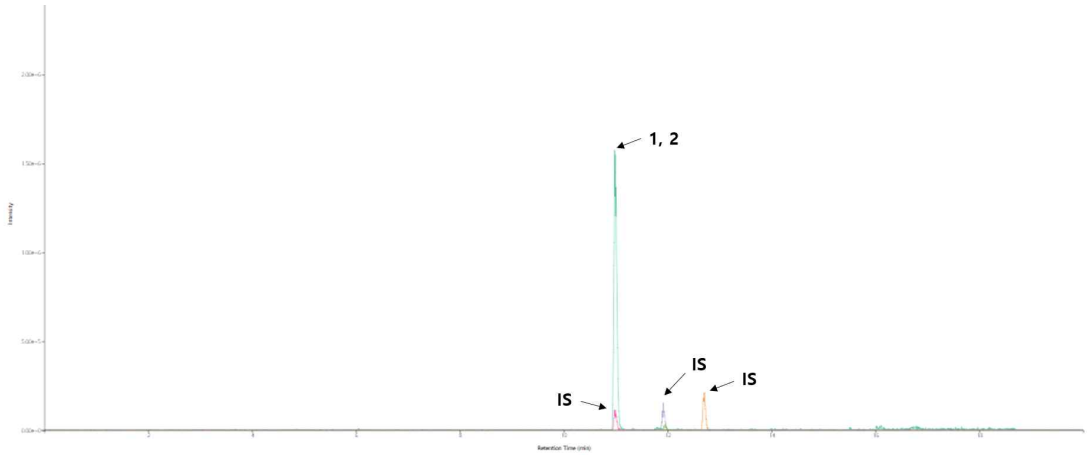
Peak ID

1. Perfluorooctanoic acid(10.97 min)
2. Ammonium pentadecafluorooctanoate Pentadecafluorooctanoic acid ammonium salt(10.97 min)
3. Tetraconazole(11.61 min)
4. Perfluorononanoic acid(11.91 min)
5. Ammonium perfluorononanoate(11.91 min)
6. Sodium heptadecafluorononanoate(11.91 min)
7. Perfluorooctanesulfonic acid(11.96 min)
8. Potassium perfluorooctanesulfonate(11.96 min)
9. Lithium perfluorooctanesulfonate(11.96 min)
10. Nonadecafluorodecanoic acid(12.70 min)
11. Ammonium nonadecafluorodecanoate(12.70 min)
12. Sodium nonadecafluorodecanoate(12.70 min)

Internal standard(IS). Perfluoro-n-[1,2,3,4-13C4]octanoic acid(10.99 min)
Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid(11.91 min)
Sodium perfluoro-1-[1,2,3,4-13C4] octanesulfonate(11.95 min)
Perfluoro-n-[1,2-13C2]dodecanoic acid(12.70 min)

※ Since the perfluorooctanesulfonic acid has an isomer structure, several peaks were confirmed when analyzing the standard solution.

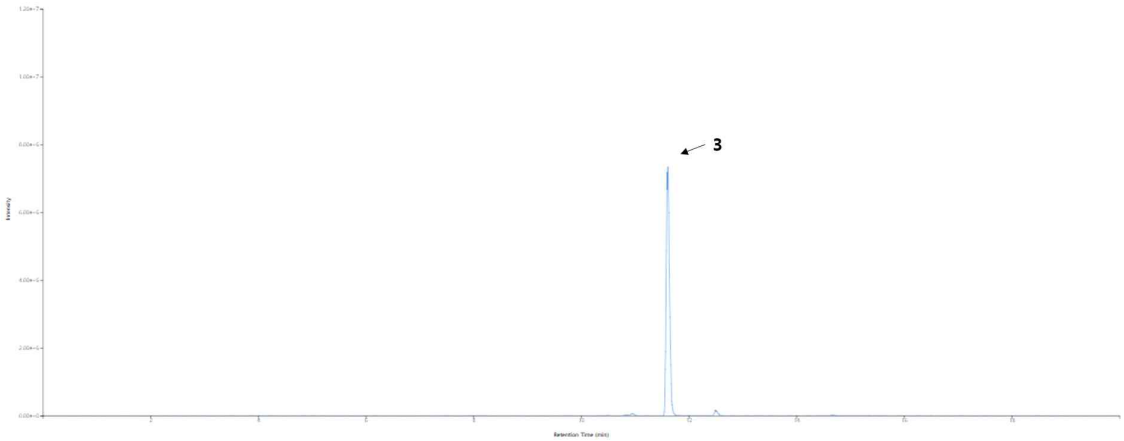
- Extracted Ion Chromatogram




Peak ID

1. Perfluorooctanoic acid(10.97 min)
2. Ammonium pentadecafluorooctanoate Pentadecafluorooctanoic acid ammonium salt(10.97 min)

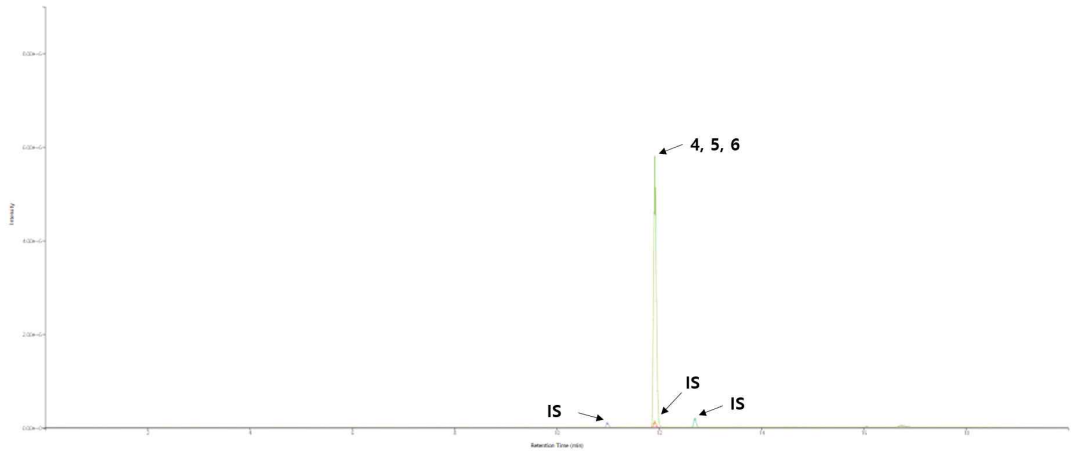
Internal standard(IS). Perfluoro-n-[1,2,3,4-13C4]octanoic acid(10.99 min)
Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid(11.91 min)
Sodium perfluoro-1-[1,2,3,4-13C4] octanesulfonate(11.95 min)
Perfluoro-n-[1,2-13C2]dodecanoic acid(12.70 min)




Peak ID

3. Tetraconazole(11.61 min)

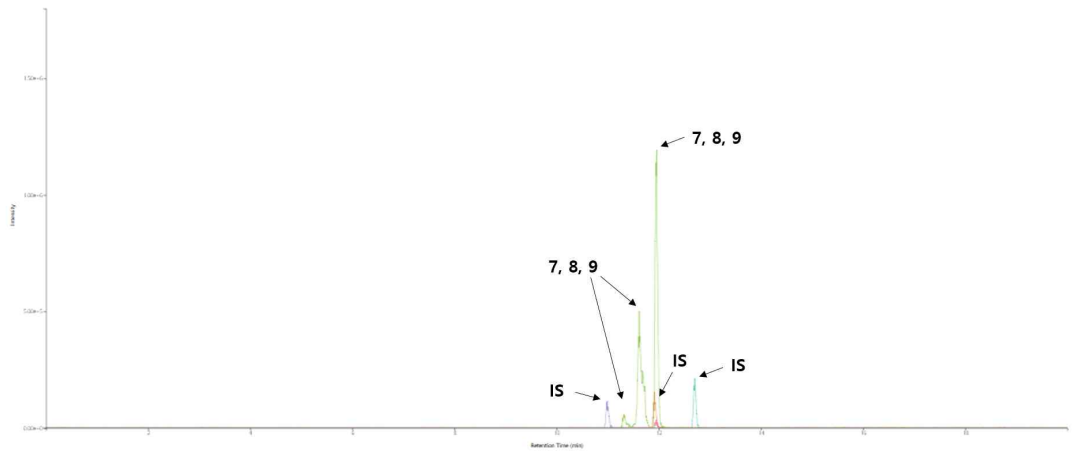
Internal standard(IS). Perfluoro-n-[1,2,3,4-13C4]octanoic acid(10.99 min)
Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid(11.91 min)
Sodium perfluoro-1-[1,2,3,4-13C4] octanesulfonate(11.95 min)
Perfluoro-n-[1,2-13C2]dodecanoic acid(12.70 min)




## Peak ID

4. Perfluorononanoic acid(11.91 min)
5. Ammonium perfluorononanoate(11.91 min)
6. Sodium heptadecafluorononanoate(11.91 min)

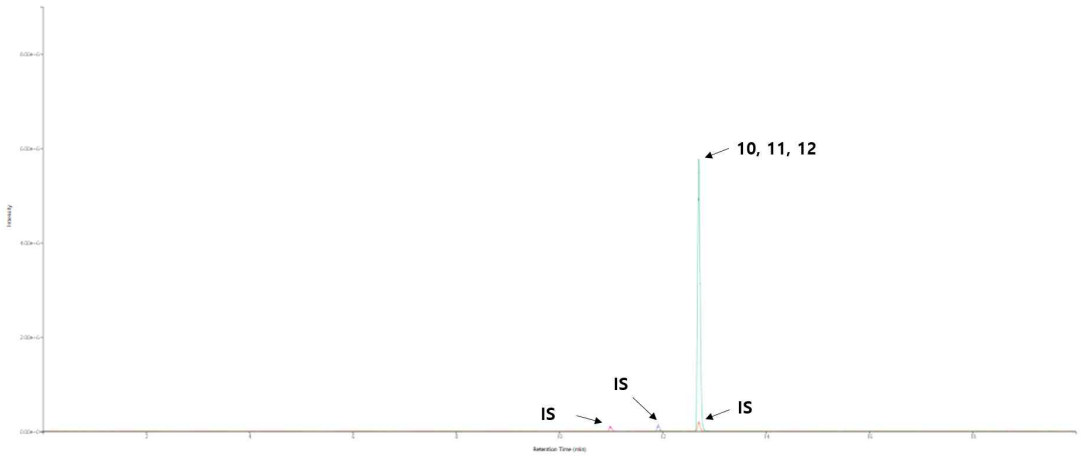
Internal standard(IS). Perfluoro-n-[1,2,3,4-13C4]octanoic acid(10.99 min)
Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid(11.91 min)
Sodium perfluoro-1-[1,2,3,4-13C4] octanesulfonate(11.95 min)
Perfluoro-n-[1,2-13C2]dodecanoic acid(12.70 min)




## Peak ID

7. Perfluorooctanesulfonic acid(11.96 min)
8. Potassium perfluorooctanesulfonate(11.96 min)
9. Lithium perfluorooctanesulfonate(11.96 min)

Internal standard(IS). Perfluoro-n-[1,2,3,4-13C4]octanoic acid(10.99 min)
Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid(11.91 min)
Sodium perfluoro-1-[1,2,3,4-13C4] octanesulfonate(11.95 min)
Perfluoro-n-[1,2-13C2]dodecanoic acid(12.70 min)



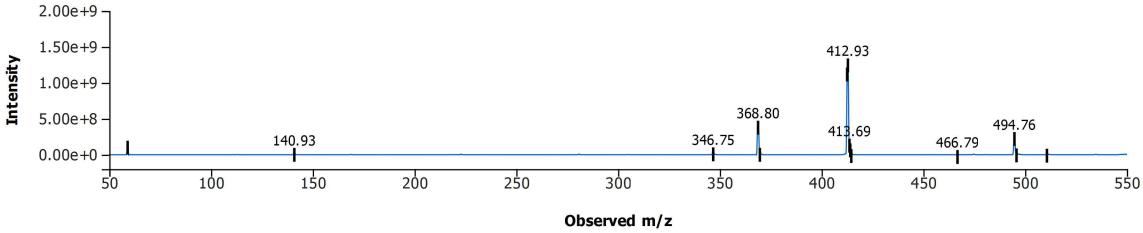

## Peak ID

10. Nonadecafluorodecanoic acid(12.70 min)
11. Ammonium nonadecafluorodecanoate(12.70 min)
12. Sodium nonadecafluorodecanoate(12.70 min)

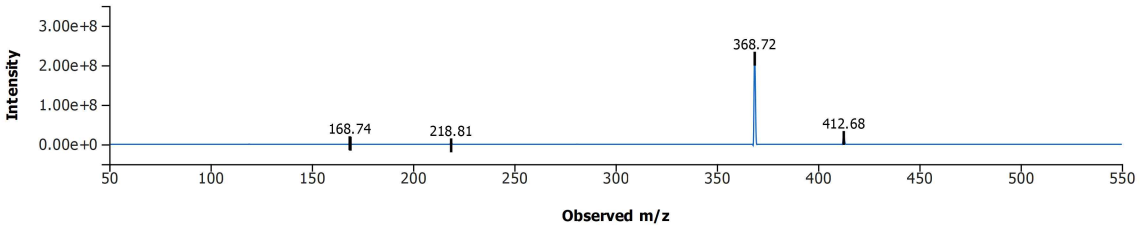
Internal standard(IS). Perfluoro-n-[1,2,3,4-13C4]octanoic acid(10.99 min)
Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid(11.91 min)
Sodium perfluoro-1-[1,2,3,4-13C4] octanesulfonate(11.95 min)
Perfluoro-n-[1,2-13C2]dodecanoic acid(12.70 min)

- Mass Spectrum

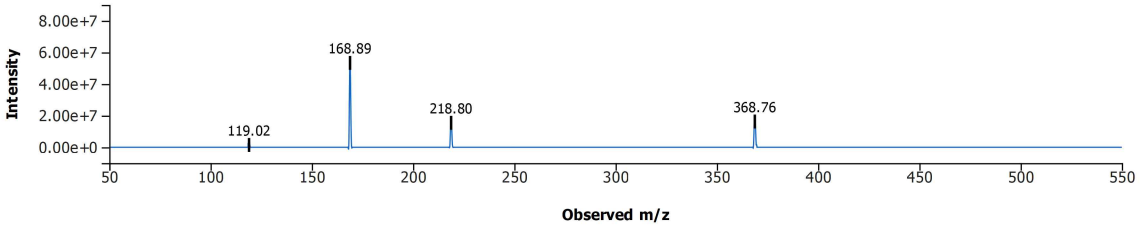
1, 2. Perfluorooctanoic acid and its salts(Ammonium)




Precursor ion

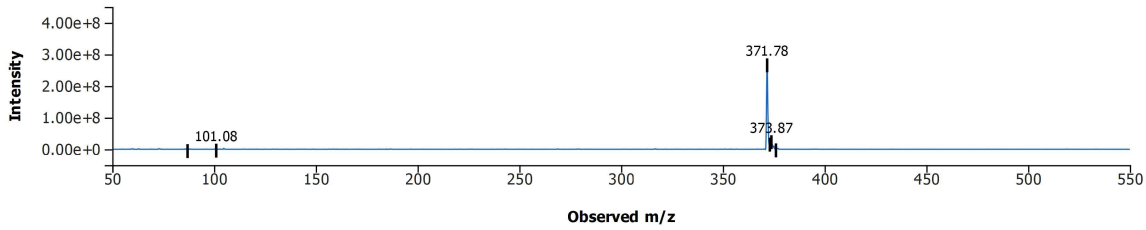



Product ion

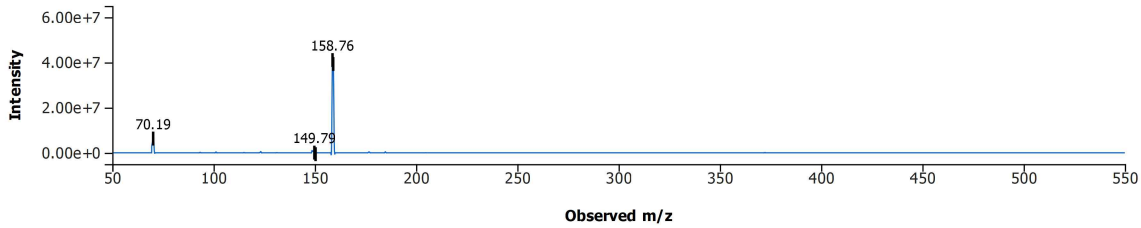



Product ion

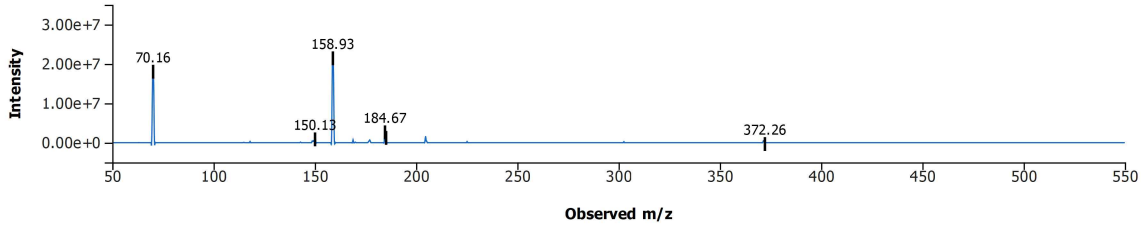
## 3. Tetraconazole




Precursor ion

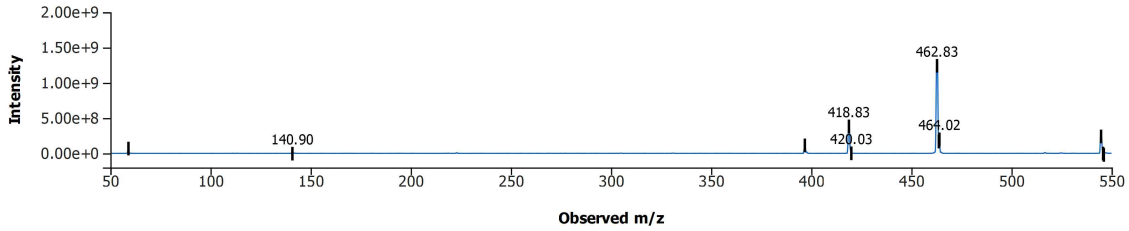



Product ion

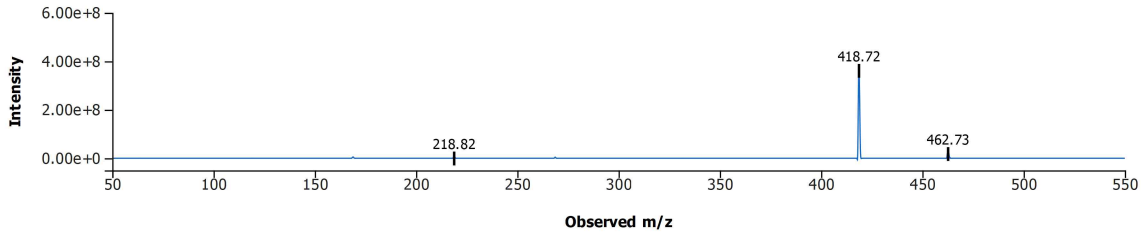



Product ion

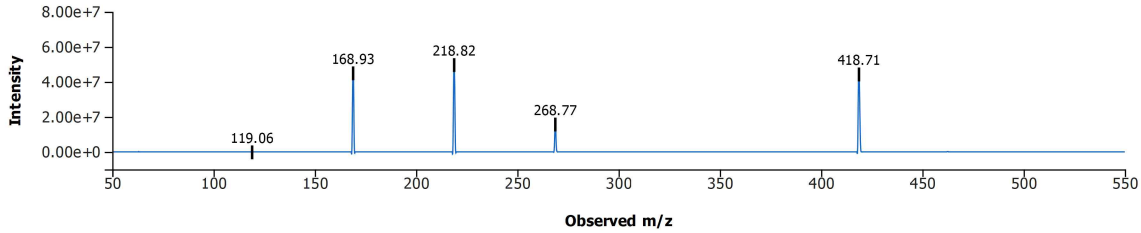
4, 5, 6. Perfluorononanoic acid and its salts(Ammonium and Sodium)




Precursor ion

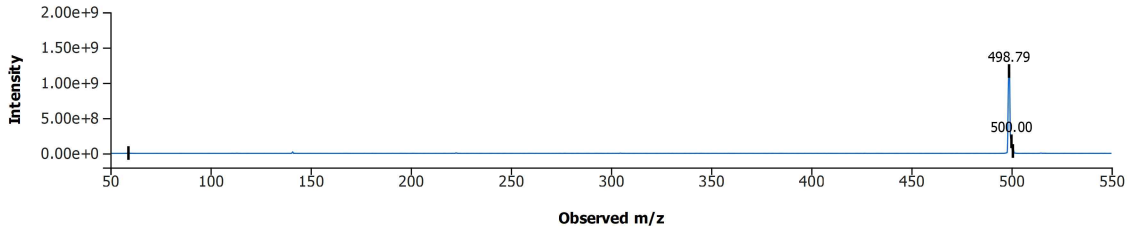



Product ion

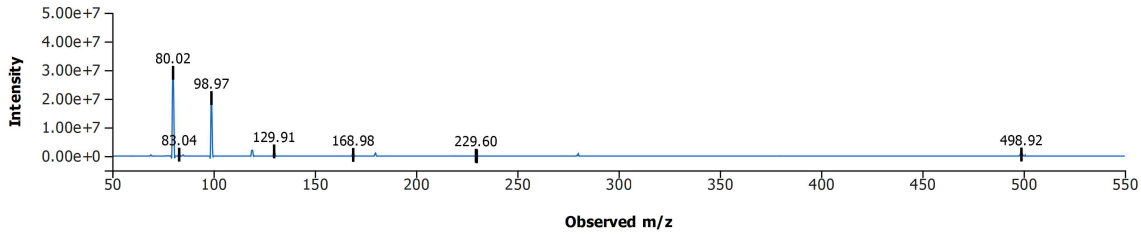



Product ion

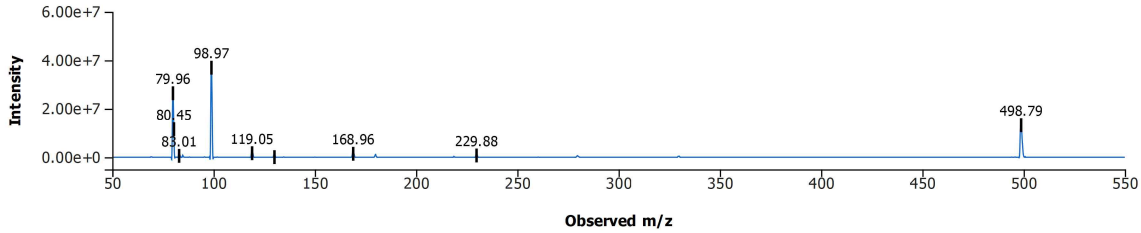
7, 8, 9. Perfluorooctanesulfonic acid and its salts(Potassium perfluorooctanesulfonate and Lithium)




Precursor ion

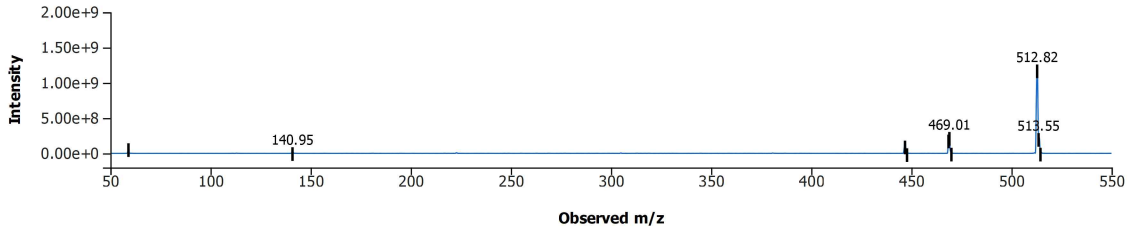



Product ion

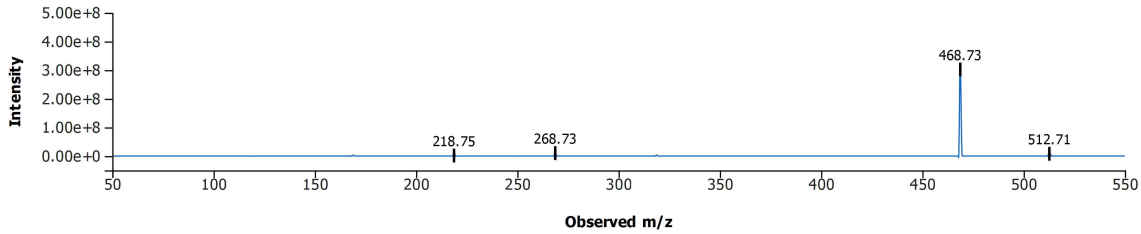



Product ion

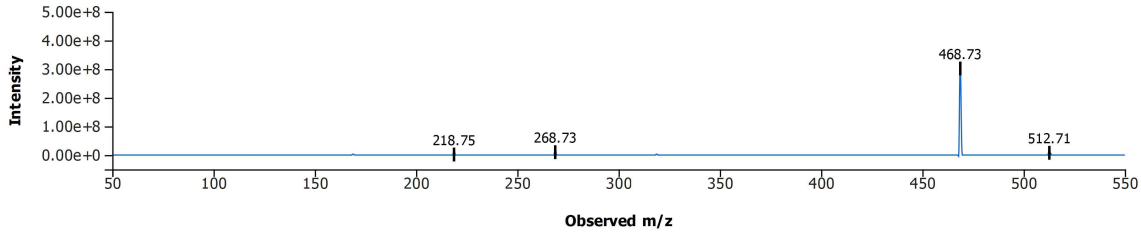
## 10, 11, 12. Nonadecafluorodecanoic acid(Ammonium and Sodium)




Precursor ion




Product ion




Product ion

## References


1) Guidance for validation of test methods for pharmaceuticals, Ministry of Food and Drug Safety, 2015.

2) Screening and validation of a pretreatment method for analyzing emerging fluorinated compounds in industrial wastewater samples. *Ki Yong Kim et al. Journal of environmental analysis, health and toxicology.* 23(2) 90-100, 2020.

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4) Fluorinated compounds in north american cosmetics. Heather D. Whitehead et al. *Environmental Science & Technology Letters.* 8, 538-544, 2021.

5) Method development and validation of per- and polyfluoroalkyl substances in foods from FDA's total diet study program. Susan Genualdi et al. *Journal of Agricultural and Food Chemistry.* 69(20), 5599-5606, 2021.

6) Per- and polyfluoroalkyl substances and fluorine mass balance in cosmetic products from the Swedish market: implications for environmental emissions and human exposure. Lara Schultes et al. *Environmental Science: Processes & Impacts.* 20, 1680-1690, 2018.

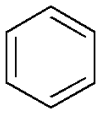
7) Sorption of perfluoroalkyl substances in sewage sludge. Jelena Milinovie et al. *Environmental Science and Pollution Research.* 2016, 23, 8839-8348

8) Structural identification of isomers present in technical perfluorooctane sulfonate by tandem mass spectrometry. Ingrid Langlois et al. *Rapid Communication in Mass Spectrometry.* 20(5), 844-850, 2006.

# 2 Benzene

## Chemical information

### Benzene



- **Molecular formula:** $C_6H_6$
- **Molecular weight:** 78.11
- **CAS number:** 71-43-2

## Assay

- **Procedure**

In a 50-mL volumetric flask, weigh 0.5 g of the sample, make it up to 30 mL using acetone, and sonicate the mixture to disperse the sample evenly, and make it up to 50 mL using acetone. Filter the resulting soultion through a membrane filter and use this solution as the sample solution. In a 10-mL volumetric flask, weigh 10 mg of the benzene, make it up to 10 mL using acetone, and use this solution as the standard stock solution(1000 μg/mL). Transfer 1 mL of the standard stock solution and make it up to 100 mL using acetone. In a 5 different glass vials, transfer 0.1, 0.2, 0.5, 1, and 2 mL of this standard solution, respectively, make it up to 10 mL using acetone, and use this solution as the standard solution.

Analyze the prepared standard solutions and sample solution as directed under gas chromatography-mass spectrometry. The sample amount or dilution factor may be adjusted within the range of the calibration curve, if necessary.

- **Chromatographic system**

  **Mode:** Gas chromatography−mass spectrometry

  **Column:** DB−WAX (0.25 μm, 0.25 x 60 mm) or equivalent column

  **Column temperature:** 40℃ (2min) → 8℃/min → 200℃ (5min)

  **Mobile phase:** Helium

  **Flow rate:** 1.0 mL/min

  **Injection mode:** Split (20 : 1)

  **Injection volume:** 1 μL

  **Injector temperature:** 240℃

  **Solvent delay:** 1 minutes

  **Detector:** Mass spectrometry

  **Ionization mode:** EI mode(Positive)

  **Electron energy:** 70 eV

  **Interface temperature:** 240℃

  **Ion source temperature:** 230℃

  **Quadrupole temperature:** 150℃

  **Mass spectrometry mode:** SIM mode

| Name | Selected ion (m/z) |
|---|---|
| Benzene | 78, 77, 51 |

Calculate the percentage amount of benzene using the following expression:

$$\text{Amount (\%) of benzene} = C \times \frac{V}{S \times 10000} \times D$$

C : Concentration of benzene calculated from calibration curve (μg/mL)

V : Final volume of the sample solution (mL)

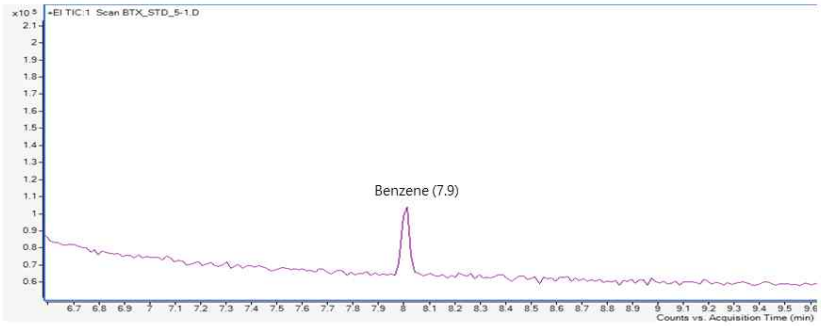
S : Amount of sample (g)

10000 : Conversion factor

D : Dilution factor

# Chromatogram & Spectrum

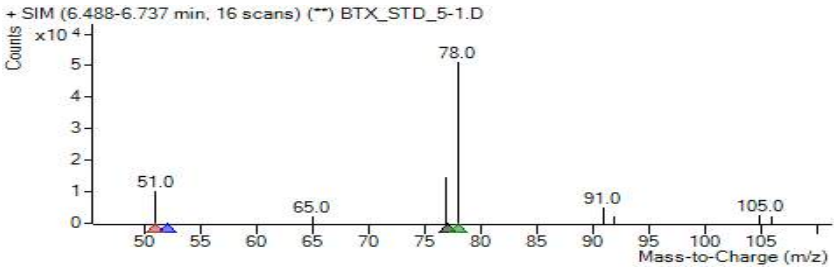
- Chromatogram




**Peak ID**

Benzene(7.9 min)

- Spectrum




Benzene

## References

1) Guidance for validation of test methods for pharmaceuticals, Ministry of Food and Drug Safety, 2015.

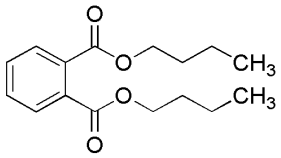
2) Jie Ji, Chunhui Deng, Wenwen Shen, Xiangmin Zhang, Field analysis of benzene, toluene, ethylbenzene and xylene in water by portable gas chromatography–microflame ionization detector combined with headspace solid–phase microextraction, *Talanta*, 69, 894–899, 2006.

3) H. Jurdakova, R. Kubinec, M. Jurcisinova, Z. Krkosova, J. Blasko, I. Ostrovsky, L. Sojak, V.G. Berezkin, Gas chromatography analysis of benzene, toluene, ethylbenzene and xylenes using newly designed needle trap device in aqueous samples, *Journal of Chromatography A*, 1194, 161–164, 2008.

# 3 Phthalates

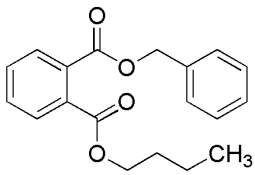
## Chemical information

### Dibutyl phthalate



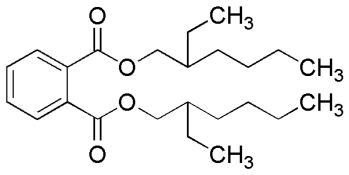
- Molecular formula: $C_{16}H_{22}O_4$
- Molecular weight: 278.34
- CAS number: 84-74-2

### Benzyl butyl phthalate



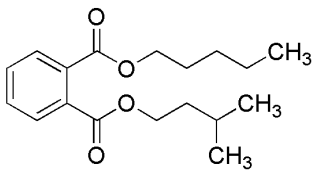
- Molecular formula: $C_{19}H_{20}O_4$
- Molecular weight: 312.36
- CAS number: 85-68-7

## Diethyl hexyl phthalate



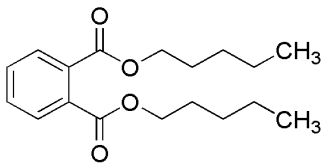
- Molecular formula: $C_{24}H_{38}O_4$
- Molecular weight: 390.56
- CAS number: 117-81-7

## Isopentyl pentyl phthalate



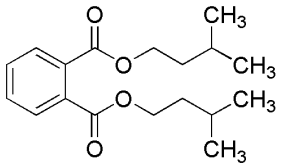
- Molecular formula: $C_{18}H_{26}O_4$
- Molecular weight: 306.40
- CAS number: 776297-69-9

## Di-n-pentyl phthalate



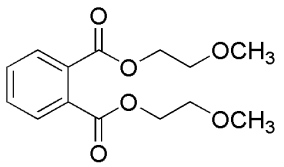
- Molecular formula: $C_{18}H_{26}O_4$
- Molecular weight: 306.40
- CAS number: 131-18-0

## Diisopentyl phthalate



- Molecular formula: $C_{18}H_{26}O_4$
- Molecular weight: 306.40
- CAS number: 605-50-5

## Bis(2-methoxyethyl)phthalate



- Molecular formula: $C_{14}H_{18}O_6$
- Molecular weight: 282.29
- CAS number: 117-82-8

## Permissible detection limit

If it is confirmed through objective data that the substances have originated unintentionally from sources such as packing materials during the manufacturing or storing processes, and their complete removal is not technically possible, the permissible detection limit for the substances shall be as follows:

**Phthalates (applicable only to dibutyl phthalate, butyl benzil phthalate, and diethyl hexyl phthalate): The net total shall be no greater than 100 μg/g.**

* 「Regulations on the Safety Standards, etc. of Cosmetics (Ministry of Food and Drug Safety Notice No. 2024-9)」

## Assay

- General Precautions

The glassware used in analytical operations have to be cleaned with acetone one time and n-hexane two times and then, sufficiently dried at 105℃.

- Procedure 1 (Gas chromatography)

* Applicable to dibutyl phthalate, butyl benzyl phthalate and ethyl hexyl phthalate.

In a glass vial, weigh 1.0 g of the sample, add 0.5 mL of the internal standard solution*, and 5 mL of the mixture of hexane and acetone(8:2). Sonicate the mixture to disperse the sample evenly, make it up to 10 mL using the mixture of hexane and acetone(8:2), centrifuge this solution, and use this solution as the sample solution. In a glass vial, weigh 20.0 mg of the dibutyl phthalate, butyl benzyl phthalate and diethyl hexyl phthalate, respectively, make it up to 20 mL using the mixture of hexane and acetone(8:2), and use this solution as the standard stock solution(1000 μg/mL). In 6 different glass vials, transfer 0.01, 0.05, 0.1, 0.5, 1, and 2.5 mL of the standard stock solution, respectively, add 5 mL of the internal standard solution* to each flask, make it up to 100 mL using the mixture of hexane and acetone(8:2), and use this solution as the standard solution.

Analyze the prepared standard solutions and sample solution following the internal standard method of the gas chromatography described in the 「Korean Functional Cosmetics Codex」 General Tests. The sample amount or dilution factor may be adjusted within the range of the calibration curve, if necessary.

*Internal standard solution: In a glass vial, weigh 10 mg of benzylbenzoate and make it up to 100 mL using the mixture of hexane and acetone(8:2).

- Procedure 2 (Gas chromatography−mass spectrometry)

  * Applicable to dibutyl phthalate, butyl benzyl phthalate, ethyl hexyl phthalate, n-pentyl-isopentyl phthalate, di-n-pentyl phthalate, diisopentyl phthalate and bis(2-methoxyethyl) phthalate.

In a glass vial, weigh 1.0 g of the sample, add 10 mL of the internal standard solution*, sonicate the mixture to disperse the sample evenly, and centrifuge this solution at 5000 rpm for 10 minutes in room temperation. Transfer 5 mL of this solution to a glass vial, make it up to 10 mL using the internal standard solution*, filter the resulting soultion through a membrane filter with a pore size of 0.2 μm, and use this solution as the sample solution. In a glass vial, weigh 20.0 mg of the dibutyl phthalate, butyl benzyl phthalate, diethyl hexyl phthalate, n−pentyl−isopentyl phthalate, di−n−pentyl phthalate, diisopentyl phthalate and bis(2−methoxyethyl) phthalate, respectively, make it up to 20 mL using the internal standard solution*, and use this solution as the standard stock solution(1000 μg/mL). Transfer 1 mL of the standard stock solution and make it up to 100 mL using the internal standard solution*(10 μg/mL). In a 6 different glass vials, transfer 0.2, 0.5, 1, 2, 4, and 8 mL of this standard solution, respectively, make it up to 10 mL using the internal standard solution*, and use this solution as the standard solution.

Analyze the prepared standard solutions and sample solution as directed under gas chromatography−mass spectrometry. The sample amount or dilution factor may be adjusted within the range of the calibration curve, if necessary.

*Internal standard solution: In a glass vial, weigh 10 mg of anthracene−d10 and make it up to 100 mL using the mixture of hexane and acetone(8:2). Trnasfer 10 mL of this solution and make it up to 1000 mL using the mixture of hexane and acetone(8:2).

- **Chromatographic system 1**

  **Mode:** Gas chromatography

  **Column:** Agilent DB-1701 (0.25 μm, 0.25 mm x 30 m) or equivalent column

  **Oven temperature:** 150℃ (2min) → 10℃/min → 260℃ (15min)

  **Injection mode:** Split (10 : 1)

  **Mobile phase:** Nitrogen

  **Flow rate:** 1 mL/min

  **Inlet temperature:** 250℃

  **Detector temperature:** 280℃

  **Detector:** Flame ionization detector(FID)

- **Chromatographic system 2**

  **Mode:** Gas chromatography-mass spectrometry

  **Column:** Agilent DB-5MS (0.25 μm, 0.25 x 30 mm) or equivalent column

  **Column temperature:** 110℃ (0.5min) → 20℃/min → 300℃ (3min)

  **Mobile phase:** Helium

  **Flow rate:** 1.0 mL/min

  **Injection mode:** Splitless

  **Injector temperature:** 280℃

  **Solvent delay:** 5 minutes

  **Detector:** Mass spectrometry

  **Ionization mode:** EI mode(Positive)

  **Electron energy:** 70 eV

  **Interface temperature:** 280℃

  **Ion source temperature:** 230℃

  **Quadrupole temperature:** 150℃

  **Scane range:** 50~300 amu

  **Mass spectrometry mode:** SIM mode

| Name | Selected ion (m/z) |
|---|---|
| Dibutyl phthalate | 149.0, 150.0, 223.1 |
| Butyl benzyl phthalate | 149.0, 91.1, 206.1 |
| Diethyl hexyl phthalate | 149.0, 167.0, 57.1 |
| n-Pentyl-isopentyl phthalate | 149.0, 71.1, 237.0 |
| Di-n-pentyl phthalate | 149.0, 105.1, 237.1 |
| Diisopentyl phthalate | 149.0, 71.1, 237.1 |
| Bis(2-methoxyethyl) phthalate | 59.1, 58.1, 149.0 |
| Anthracene-d10(Internal standard) | 188.1, 187.1, 189.1 |

Calculate the percentage amount of each ingredients using the following expression:

$$\text{Amount (\%) of each ingredients} = C \times \frac{V}{S \times 10000} \times D$$

C : Concentration of each ingredients calculated from calibration curve (μg/mL)

V : Final volume of the sample solution (mL)

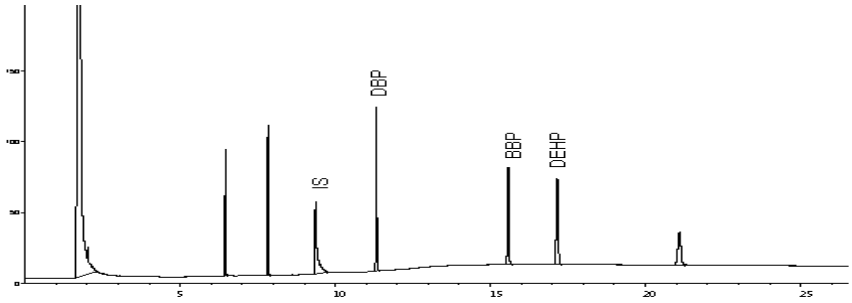
S : Amount of sample (g)

10000 : Conversion factor

D : Dilution factor

# Chromatogram & Spectrum

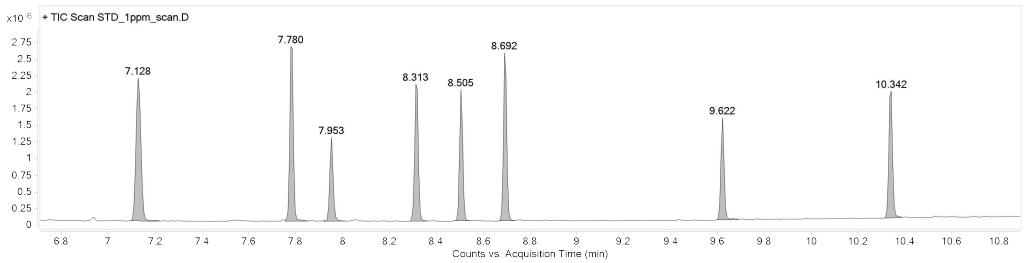
- Chromatogram (Gas chromatography)




Peak ID

1. Benzylbenzoate(Internal standard)
2. Dibutyl phthalate
3. Butyl benzyl phthalate
4. Diethyl hexyl phthalate

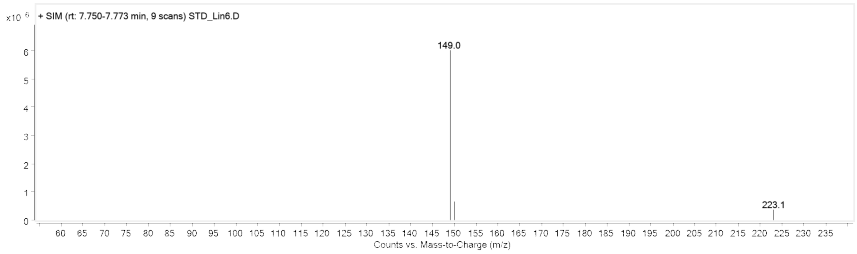
- Chromatogram (Gas chromatography-mass spectrometry)



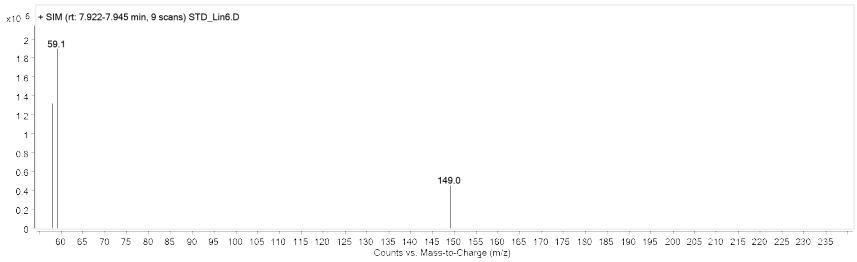

Peak ID

1. Anthracene-d10(7.1 min, Internal standard)
2. Dibutyl phthalate(7.8 min)
3. Bis(2-methoxyethyl) phthalate(7.9 min)
4. Diisopentyl phthalate(8.3 min)
5. n-Pentyl-isopentyl phthalate(8.5 min)
6. Di-n-pentyl phthalate(8.7 min)
7. Butyl benzyl phthalate(9.6 min)
8. Diethyl hexyl phthalate(10.3 min)

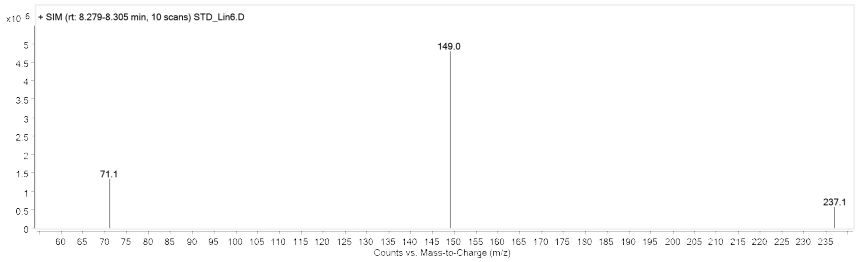
- Spectrum (Gas chromatography-mass spectrometry)



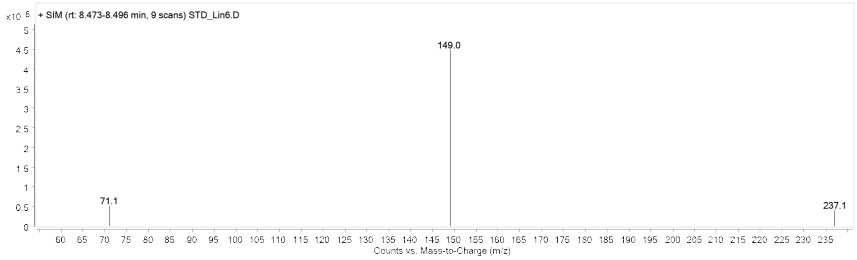

Dibutyl phthalate



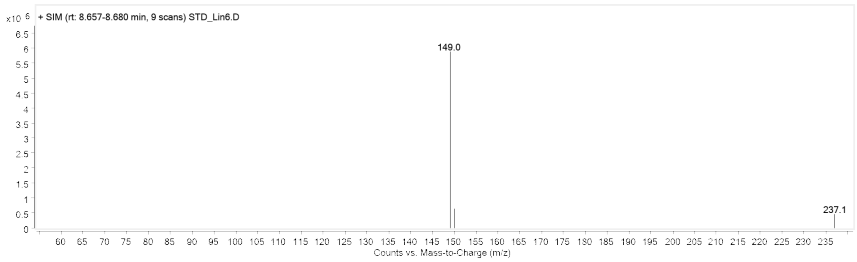

Bis(2-methoxyethyl) phthalate



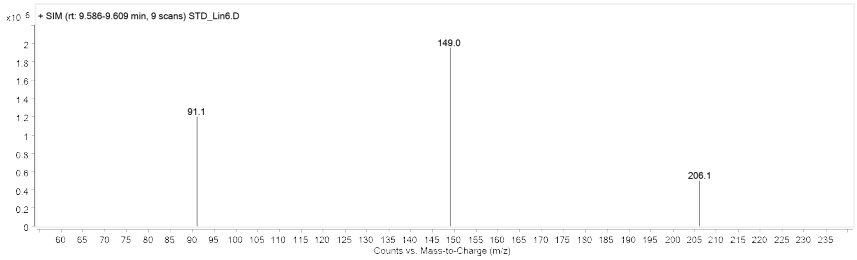

Diisopentyl phthalate



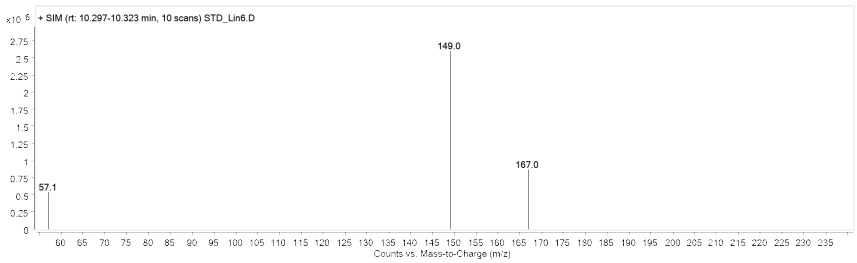

n-Pentyl-isopentyl phthalate




Di-n-pentyl phthalate




Butyl benzyl phthalate




Diethyl hexyl phthalate

## References

1) Guidance for validation of test methods for pharmaceuticals, Ministry of Food and Drug Safety, 2015.

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# Analytical Methods of Cosmetic Ingredients in Cosmetic Products


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|---|---|
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